Nicotinamide adenine dinucleotide: beyond a redox coenzyme

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ADP-ribosylation using nicotinamide adenine dinucleotide (NAD^+) is an important type of enzymatic reaction that affects many biological processes. A brief introductory review is given here to various ADP-ribosyltransferases, including poly(ADP-ribose) polymerase (PARPs), mono(ADP-ribosyl)-transferases (ARTs), NAD^+-dependent deacetylases (sirtuins), tRNA 2′-phosphotransferases, and ADP-ribosyl cyclases (CD38 and CD157). Focus is given to the enzymatic reactions, mechanisms, structures, and biological functions.

Introduction

Nicotinamide adenine dinucleotide (NAD^+, and the reduced form NADH, Fig. 1) is familiar to most chemists and biochemists since it has long been recognized as an important small molecule cofactor/coenzyme required for many cellular oxidases and reductases. As a redox coenzyme, it shuttles between the oxidized form (NAD^+) and the reduced form (NADH), as shown in Fig. 1, but the total concentration remains constant. As more cellular secrets are revealed by scientific research, it turns out NAD^+ has more functions in addition to serving as a redox coenzyme.[1,2] There are several types of enzymes that use NAD^+ as a co-substrate and consume NAD^+ in the enzymatic reactions. Some of the NAD^+-consuming enzymes have attracted great interest recently because of their unique chemistry and important biological functions.

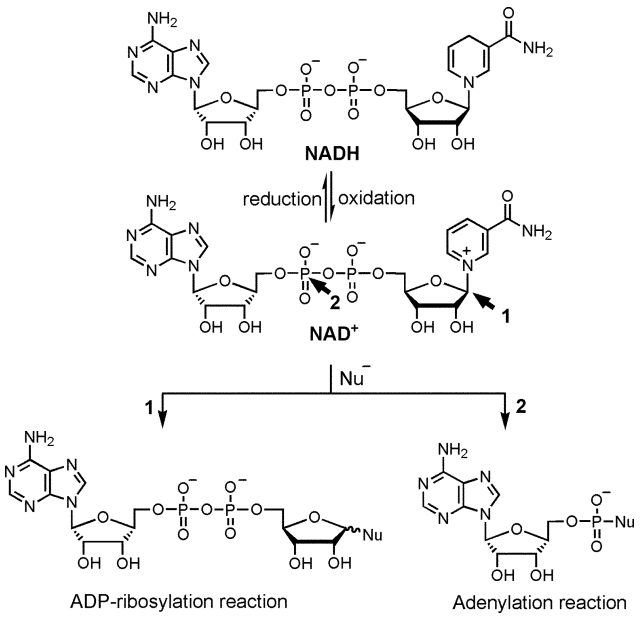

Fig. 1 Enzymatic reactions that use NAD^+. NAD^+ is mainly involved in three types of reactions: reduction–oxidation, ADP-ribosylation (pathway 1), and adenylation (pathway 2). Oxidation–reduction does not consume NAD^+ because the compound just shuttles between oxidized and reduced forms. Adenylation and ADP-ribosylation both break down NAD^+ and therefore consume NAD^+. Enzymes catalyzing ADP-ribosylation reactions are the focus here.

NAD^+ has two bonds of relatively high energy, the *N*-glycosidic bond involving nicotinamide and the pyrophosphate bond. Most NAD^+-consuming enzymatic reactions break the *N*-glycosidic bond (ADP-ribosylation reactions, Fig. 1), although breaking of the pyrophosphate bond (adenylation reactions, Fig. 1) also occurs. For example, NAD^+-dependent DNA ligases use NAD^+ to form an adenylate-ligase covalent intermediate, which is capable of transferring the adenosine 5′-monophosphate (AMP) group to the 5′-phosphate of nicked DNA ends to allow DNA ligation with release of free AMP.[3,4] NAD^+-dependent DNA ligases are essential for many bacteria species, but are not present in eukaryotes, making them attractive antibiotic targets.[5]

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The NAD^+-consuming ADP-ribosyltransferases are the focus of this manuscript. These enzymes can be categorized into several classes based on the targets they modify: *(1) ADP-ribosyltransferases that modify proteins; (2) ADP-ribosylstranferases that modify nucleic acids; (3) ADP-ribosyltransferases that modify small molecules.* Each class consists of several different subclasses based on structure and activity. Below I will briefly summarize these enzymes and highlight some important unresolved questions. Addressing these questions will likely require a joint effort from people specializing in different areas, including organic chemists, biochemists, biophysical chemists, and biologists. The purpose of this manuscript is not to extensively review all the literature for these NAD^+-utilizing enzymes, but rather to introduce a fascinating research area to those (especially graduate students and postdocs with a chemistry background) that are relatively unfamiliar to, but might become interested in ADP-ribosylation.

ADP-ribosyltransferases that modify proteins

ADP-ribosyltransferases that catalyze protein posttranslational modifications are currently being studied extensively. By modifying different substrate proteins and thus changing the properties of the substrate proteins, these enzymes execute their biological functions in controlling/regulating various biological processes. Based on the reactions they catalyze, there are three types of ADP-ribosyltransferases in this category: poly(ADP-ribose) polymerases,[6–9] mono(ADP-ribosyl)transferases,[10–12] and NAD^+-dependent deacetylases.[13,14]

Poly(ADP-ribose) polymerases

Poly(ADP-ribosyl)ation, a modification mainly found in eukaryotes, is the covalent addition of multiple (up to several hundred) ADP-ribose groups to proteins (Fig. 2A). The first ADP-ribosyl group is typically added to the carboxylate side chain of Glu or Asp residues on the substrate protein, followed by the addition of more ADP-ribosyl groups to the 2-OH groups of the two ribose rings, leading to a long and branched poly(ADP-ribose) chain (PAR, Fig. 2A). The ADP-ribosyl linkage in PAR was determined to be in the α configuration, opposite to that of the substrate NAD^+.[15] The enzymes that catalyze the reaction are termed poly(ADP-ribose) polymerase, or PARPs. The most abundant and the best studied PARP is PARP-1, a protein of 113 kDa.[6] PARP-1 has three domains, the N-terminal DNA-binding domain that consists of two zinc fingers, a BRCT auto-modification domain in the middle, and the catalytic domain at the C-terminus (Fig. 2B). The crystal structures of several PARP catalytic domains have been reported.[16–18] Fig. 2C shows the structure of the catalytic domain of chicken PARP-1 with ADP bound (PDB 1A26).[17] By structural alignment with diphtheria toxin catalytic domain with an NAD^+ bound (PDB 1TOX),[19] the NAD^+ binding site and the acceptor site have been suggested (see the green stick representation of NAD^+ and ADP in Fig. 2C).[17] The conserved Glu998 residue shown in red is within hydrogen bond distance with the ribose ring of ADP. The role of the conserved Glu residue is presumably to deprotonate the 2-OH of the ribose of the acceptor molecule (growing PAR chain), activating it for nucleophilic attack on the NAD^+ co-substrate.[17] It is possible that the Glu residue is also involved in stabilizing the oxo-carbenium ion-like transition state or intermediate.[17]

PARP-1, the founding member of PARPs, is well known to be involved in DNA repair. PARP-1 activity is low at resting state. PARP-1 DNA-binding domain binds to DNA strand breaks generated directly or indirectly from oxidation, alkylation, base excision, and many other types of DNA damage. This interaction activates the catalytic activity of the catalytic domain up to several hundred-fold, resulting in the transfer of ADP-ribose groups to various substrate proteins, most prominently PARP-1 itself on the BRCT domain, and histones.[6] Why is PARP-1 activity needed for DNA repair? Although the detailed molecular picture is still not completely clear, several models/explanations have been proposed. It is important to bear in mind that in eukaryotes, DNA is tightly packed into nucleosomes around octamers of histones.[20] This packing has significant impact on everything that happens to DNA, such as replication, transcription, and damage repair. One model is that PARP-1-catalyzed poly(ADP-ribose) formation relaxes the chromatin structure,[21–23] to allow the DNA repair enzymes to access and repair the damaged DNA. Another model suggests that PARP-1, and the poly(ADP-ribose) formed, either covalently attached to proteins or free of proteins (generated by the hydrolase PARG),[24] could serve to recruit DNA repair enzymes and/or other proteins that are involved in the process.[6,25,26] It is possible that each model captures one certain aspect of poly(ADP-ribosylation) in DNA repair and that all of them might contribute to the biological function of PARP-1 in DNA repair (Fig. 3).

PARP-1 activity in DNA repair is a double-edged sword. If too much DNA damage occurs, PARP-1 over-stimulation leads to cell death, possibly due to depletion of cellular NAD^+ and/or the signaling functions of PAR polymer.[32,33] In contrast, PARP-1 knockout cells are resistant to large doses of DNA damaging (alkylating and oxidizing) reagents that are lethal to normal cells. This may partly explain why PARP inhibitors offer protection in many pathophysiological conditions, such as stroke and ischemia-reperfusion.

PARP-1 function, however, is not limited to DNA repair. PARP-1, the most abundant nuclear PARP protein, also has an important function in transcription regulation. In *Drosophila*, it was found that PARP-1 and poly(ADP-ribose) are associated with genes that are activated by steroids or stress. This gene activation is accompanied by local loosening of the chromatin or puffing.[34] PARP-1 catalytic activity is required for puffing, since the PARP-1 inhibitor 3-aminobenzamide inhibited puff formation and the transcription of the genes. The proposed model to explain the PARP-1 dependent puffing in *Drosophila* is that PARP-1 is associated with many regions of the chromatin, and specific signals such as steroids and stress activate PARP-1 activity in specific regions of the chromatin, which leads to the poly(ADP-ribosyl)ation of nearby proteins (for example, histones). This then causes the relaxation of local chromatin structure and enables the transcription of the genes nearby (Fig. 3).[34] This model seems reasonable, especially as it has been reported that PARP-1 can bind to different forms of undamaged DNA (for example, cruciforms, supercoiled plasmids) in addition to DNA strand breaks.[6,35] Kraus and coworkers recently reported that PARP-1 can bind to nucleosomes formed *in vitro*.[29] Similar to linker histone H1, PARP-1 binds to linker DNA where DNA exits the nucleosome. However, in the polytene chromosome of flies,

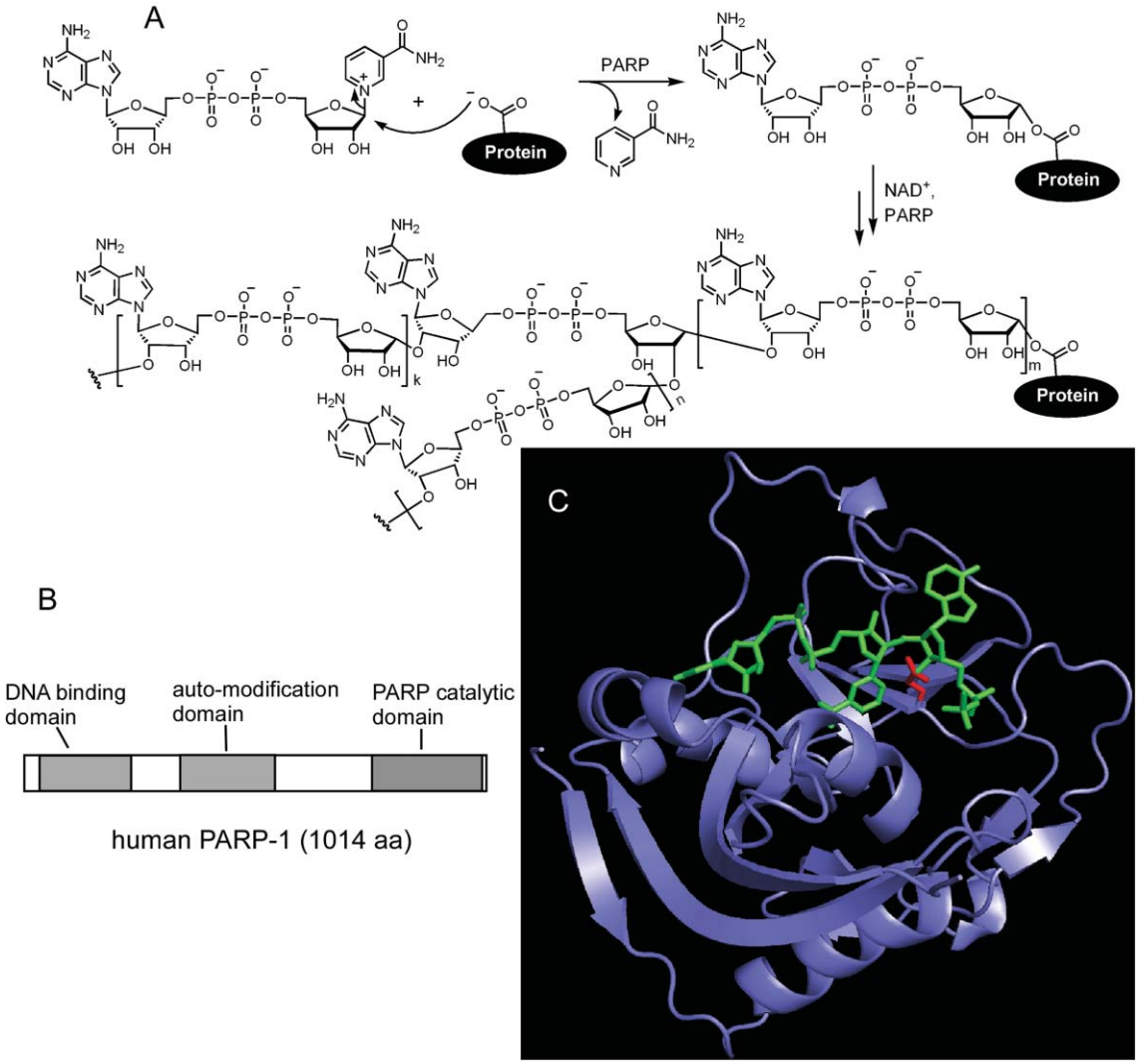

Fig. 2 The biochemical activity of PARPs. (A) PARPs catalyze the addition of multiple ADP-ribosyl groups to Glu/Asp residues of substrate proteins, giving rise to long and branched ADP-ribose polymers. (B) The domain organization of human PARP-1. The N-terminal DNA binding domain recognizes different forms of DNA and is important in regulating the catalytic activity of the C-terminal catalytic domain. (C) The crystal structure of the catalytic domain of chicken PARP-1 (generated using PDB 1A26). The active site Glu998 residue is shown in red. This Glu998 residue is likely responsible for the deprotonation of the 2-OH on the acceptor molecule (green stick representation on the right) and/or the stabilization of the oxo-carbenium ion-like transition state/intermediate formed upon leaving of nicotinamide from NAD^+ (green stick representation on the left). The NAD^+ molecule is modeled by superimposing the PARP-1 structure onto the diphtheria toxin structure (PDB 1TOX), which contains an NAD^+ molecule.[17]

PARP-1 and H1 bind to different regions of the chromatin that are not actively transcribed. The nucleosome-bound PARP-1 is catalytically active when NAD^+ molecules are present, leading to self-modification and dissociation from the nucleosome to allow RNA Pol II to access the DNA for transcription.[29] This model, in many aspects, is similar to the model proposed by Tulin and Spradling,[34] although it differs in some details. This model implies that NAD^+ is not freely available in the nucleus because otherwise PARP-1 would not be able to bind to nucleosomes. Currently, there is still no method that can reliably determine the concentration of a particular metabolite within a specific organelle inside a cell. Therefore, whether or not this model is accurate still awaits further studies. The recent report that PARP-1 and the nicotinamide mononucleotide adenylate transferase (NMNAT, the enzyme that catalyzes the last step of NAD^+ biosynthesis) interact with each other in the nucleus could lend indirect support to this model.[36] Alternatively, PARP-1 interacting proteins, such as macroH2A,[30] might inhibit its catalytic activity at resting state and the inhibiting proteins dissociate in the presence of appropriate signals.

A new twist to the function of PARP-1 in transcription regulation is the discovery that PARP-1 and DNA topoisomerase IIβ (TopoIIβ) coexist in a co-activator complex that is recruited to the *pS2* gene promoter during estrogen receptor activated transcription of *pS2*.[37] Moreover, the catalytic activities of both PARP-1 and TopoIIβ are required for the transcription activation. TopoIIβ is found to specifically introduce a double strand break in the promoter sequence, which presumably activates PARP-1 catalytic activity, leading to the replacement of linker histone H1 with high mobility group B 1/2 (HMGB1/2). However, whether H1 is poly(ADP-ribosyl)ated or not in this process

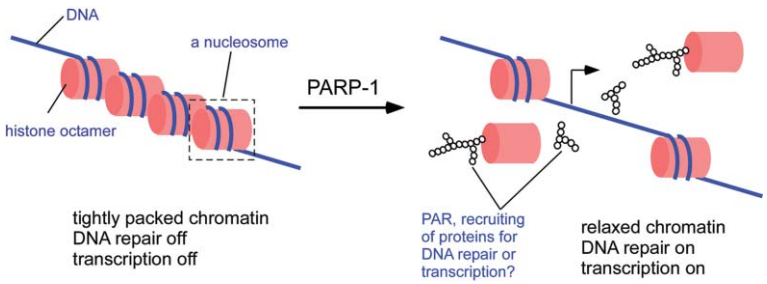

Fig. 3 PARP-1 function in DNA repair and transcription regulation. This simplified picture summarizes some of the understanding about PARP-1 function. At resting state, DNA in eukaryotic cells are packed tightly into nucleosomes, which generally blocks access to proteins involved in DNA repair and transcription. However, when DNA strand break occurs, or an upstream signal occurs (steroid hormone, for example), PARP-1 activity is activated and catalyzes the synthesis of PAR on PARP-1 and histones or other nucleosomal proteins. The enormous negative charge built up on the PAR polymer could dissociate the DNA–histones complex (PAR and DNA compete for histones). Thus the DNA becomes "naked", allowing access to the DNA repair enzymes or proteins involved in transcription. The PAR polymer could also actively recruit other proteins, such as proteins that contain macro domains.[27,28] PARP-1 binds strongly to DNA strand break and the binding somehow triggers its catalytic activity. How PARP-1 is activated during transcription regulation is not entirely clear. Results from the Kraus lab suggest that it might be due to the increase of local NAD^+ concentration[29] or the release of inhibiting proteins.[30] Results from the Rosenfeld Lab suggest that it might be due to the DNA strand break generated by TopoIIβ.[37] Alternatively, a growth factor induced signaling cascade was shown to activate PARP-1 independent of DNA.[31]

has not been determined. The biological function of TopoIIβ has been somewhat enigmatic, although it has been shown to be involved in transcriptional regulation of genes involved in cell differentiation.[38,39] The study by Rosenfeld and coworkers provided a potential molecular link among TopoIIβ, PARP-1, and transcriptional activation.[37]

The above three examples about PARP-1 and transcription regulation clearly demonstrate that PARP-1 plays important roles in the transcriptional activation of certain genes. Although the detailed molecular picture still needs to be figured out, it is clear that PARP-1's DNA binding property and catalytic activity are both important. However, it is also reported that in some transcription regulation processes, PARP-1's catalytic activity is not required.[40] Many questions need to be addressed by future studies, including the following: (i) what regions of the chromosome are bound by PARP-1 and therefore the transcription regulated by PARP-1, and how is this specificity determined? (ii) In the transcription activation process, how is PARP-1 catalytic activity regulated, what is the major acceptor for poly(ADP-ribosyl)ation, and is the acceptor determined by the specific PARP-1 activator or not? (iii) At the molecular level, how does poly(ADP-ribosyl)ation lead to transcription activation or DNA repair? Does poly(ADP-ribosyl)ation simply relax chromatin structure or do the PAR polymers actively recruit other proteins, such as proteins that contain macro domains?[27,28] (iv) Structurally, how do different forms of DNA or other signals bind to PARP-1 and activate its catalytic activity?

Another direction in the poly(ADP-ribosyl)ation field is the study of other PARP enzymes. Bioinformatic studies suggest that there are 18 PARP proteins in the human genome.[41] All the 17 relatively new PARP proteins share the conserved catalytic domain with PARP-1, but the rest of the protein sequence is very diverse. It is not known whether all of the other PARP proteins retain the catalytic activity, but many of them do.[7] If they do have protein ADP-ribosylation activity, what substrate proteins do they modify and what functions does the modification have? For most of the 17 PARPs, very little is known. One of the better known PARPs is tankyrase-1, which was shown to have two important functions. One function is to regulate telomere length maintenance by poly(ADP-ribosyl)ating a telomere-associated protein TRF-1.[42,43] TRF-1 blocks telomerase from accessing the telomere. Poly(ADP-ribosyl)ation by tankyrase-1 releases TRF-1 from telomere and therefore allows telomere elongation by telomerase. Tankyrase-1 and its catalytic activity are also required for mitosis,[44–46] although exactly why it is required is not very clear. The protein that is modified by tankyrase-1 during mitosis is NuMA, a mitotic spindle-pole protein.[45,46] Thus, among the 18 different PARPs, PARP-1 and tankyrase-1 allow us to have a peek at what important biological functions poly(ADP-ribosyl)ation could have, and what we are seeing could be just "the tip of the iceberg".

Mono(ADP-ribosyl)transferases

Mono(ADP-ribosyl)ation is the transfer of a single ADP-ribose group to proteins' side chains, typically Arg, Cys (Fig. 4A), and in rare cases also Asn and posttranslationally modified diphthamide.[10,47] The enzymatic reaction mechanism is presumably similar to that of PARP. A conserved Glu residue is also present in the active site (Fig. 4B). Therefore, the ADP-ribosyl linkage in the product is expected to be α as in poly(ADP-ribosyl)ation, although this is not determined in most cases. This type of modification was originally identified for several bacterial toxins that mono(ADP-ribosyl)ate host proteins, such as diphtheria toxin, pseudomonas exotoxin A, cholera toxin, and pertusis toxin.[10,47] The discovery of eukaryotic mono(ADP-ribosyl)transferases (ART) is fairly recent.[10,11] The first cloned ART is from a rabbit in the early 1990s.[48] Since then, 5 ARTs have been identified in humans (ART1-5, although ART2 has premature stop codons and is presumably not functional) and 6 in mice (ART1, ART2a, ART2b, and ART3-5).[10,49,50] These mammalian ARTs are ecto-enzymes because they are glycosylphosphotidylinositol (GPI)-anchored to the cell membrane, with the active sites outside the cell (Fig. 4C).[10,50] Consistent with this, hydrophobic signal sequences are found both at the N-terminus (directing translating peptide chain to ER) and C-terminus (signal for GPI attachment). One of the enzymes,

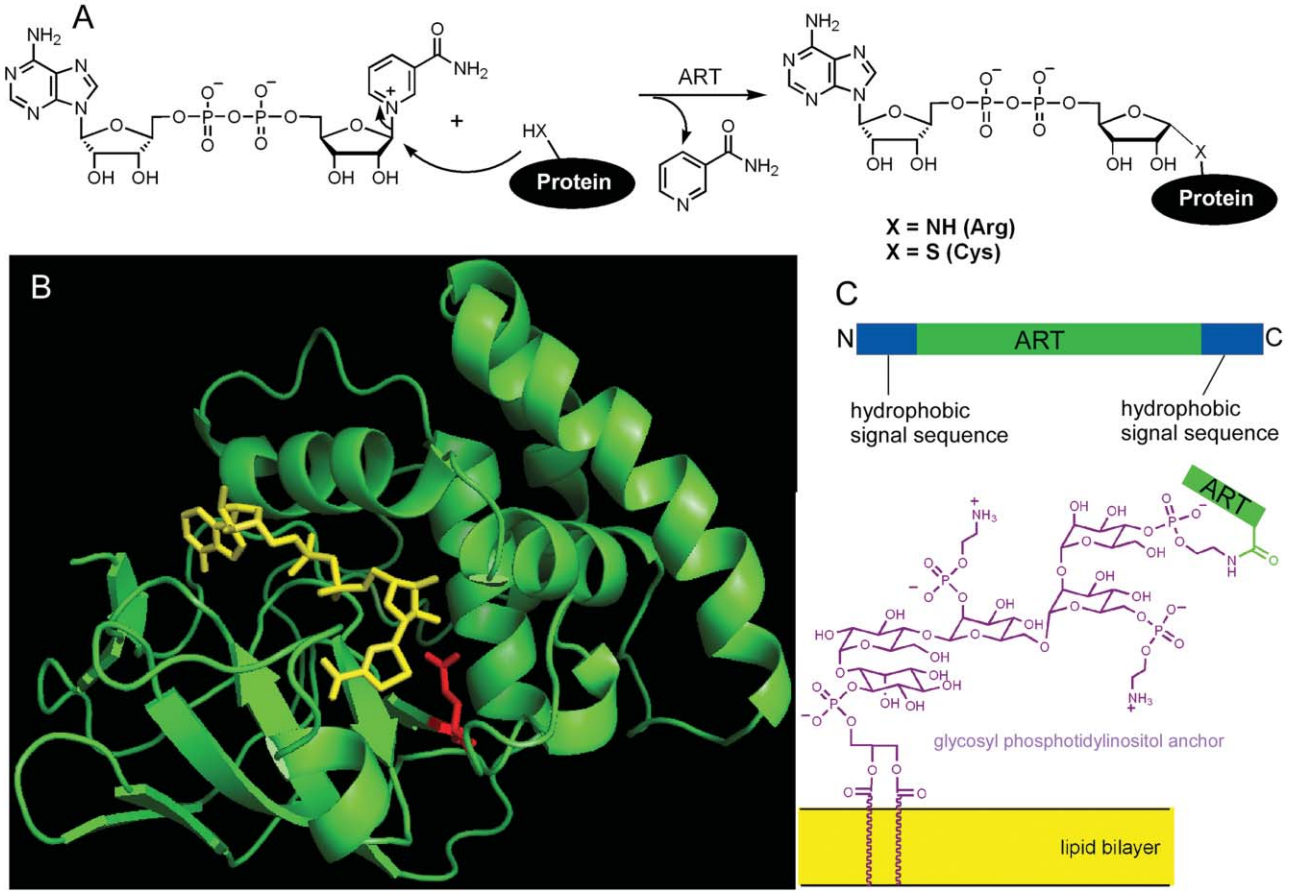

Fig. 4 The biochemical activity of ecto ARTs. (A) ARTs catalyzes the addition of a single ADP-ribosyl group to proteins, typically onto Arg or Cys residues. The glycosidic bond formed is presumably α based on the similarity of ARTs to PARPs and bacteria toxin type ADP-ribosyltransferases. (B) The structure of rat ART2 (generated using PDB 1OG1). The active site is occupied by the NAD analog shown as yellow sticks. The conserved Glu189, shown in red, likely is involved in stabilizing the oxo-carbenium ion like transition state/intermediate. (C) ARTs are ecto enzymes linked to glycosylphosphotidylinositol anchors. Most ARTs have hydrophobic signal sequences at both the N- and C-terminals. The N-terminal signal directs the translating ART polypeptides to the endoplasmic reticulum (ER) as for all other secreted proteins, and the C-terminal signal defines the GPI-anchor attachment. These GPI-anchored ARTs are then sent out for displaying on the cell surface.

ART5, lacks the C-terminal signal sequence for GPI attachment and is therefore secreted instead of membrane attached.[50]

The functions of protein ADP-ribosylation by the ecto ARTs are being studied, and a detailed molecular picture is slowly emerging. In mouse skeletal muscle cells, the expression of ART1 correlates with the transition from mononucleated, replicating myoblasts to long, multinucleated non-replicating myotubes.[51] Using ^{32}P-labeled NAD^+, mouse skeletal muscle ART1 was found to ADP-ribosylate integrin α7, which modulates its binding to the extracellular matrix protein laminin in the presence of Mn^{2+}.[52] The ADP-ribosylated integrin α7 can be processed by cellular phosphodiesterases to phosphoribosylated protein and AMP.[53] It is not known how the phosphoribosylation affects integrin α7 binding to laminin. In mouse T-cells, ART1 is found to ADP-ribosylate several proteins, including LFA-1, CD27, CD43, CD44, and CD45.[54] The ADP-ribosylation of these proteins interferes with the T-cell receptor signaling by inhibiting the T-cell receptor and co-receptors to form a functional cluster on the cell membrane.[55] ART1 on human airway epithelium cells can ADP-ribosylate defensin-1 or HNP-1 *in vitro*.[56] Consistent with this, ADP-ribosylated HNP-1 was isolated from the bronchoalveolar lavage fluid of smokers, but not of non-smokers. ADP-ribosylated HNP-1 has decreased anti-microbial activity and cytotoxicity, but can still stimulate T-cell chemotaxis and IL-8 release.[56] Other proteins have also been found to be ADP-ribosylated by ART-1 in the presence of NAD^+, including fibroblast growth factor-2 and platelet-derived growth factor-BB.[57,58] ADP-ribosylation of these growth factors seems to affect their binding to the receptors, and could therefore regulate their biological activity.

ART2 is found to be expressed on mature T-cells in mice. Interestingly, it is reported that on mouse T-cells expressing both ART2 and the purino receptor P_2X_7, NAD^+ at low μM concentrations can induce T-cell death.[59] Available evidence suggests that the NAD^+-induced cell death is *via* ART2-catalyzed P_2X_7 ADP-ribosylation.[60] P_2X_7 has been known to induce cell death under a mM concentration of ATP, which is too high and is unlikely to occur extracellularly *in vivo.*; while the low μM concentration of NAD^+ might be physiologically achievable during cell lysis. It is proposed that the ART2 and P_2X_7 dependent cell death induced by NAD^+ might regulate immune response to avoid unintended activation of bystander T-cells under conditions of massive cell lysis.[60] It should be noted that ART2 is not present in humans

because of the premature stop codon present in the human ART2 gene.[50] Whether a similar regulation exist in human T-cells is not clear now. It is possible that other ARTs, such as ART1, which is expressed in human T-cells, could have similar functions since mouse ART1, ART2, and human ART1 can all ADP-ribosylate LFA-1 when expressed in the mouse lymphoma cell line DC27.10.[61]

At present, not much is known about the functions of ART3, ART4, and ART5, since no substrate proteins have been identified yet. Therefore, one future direction is to identify the substrate proteins for these ecto ARTs. Even for ART1 and ART2, identification of new substrate proteins might offer more insight to their functions. Another question is the source of NAD^+ *in vivo* for ecto ARTs. Although intracellular concentration of NAD^+ is high (estimated to be from 100 μM to several mM), extracellular NAD^+ concentration is estimated to be less than 0.1 μM,[60] which is not enough for the ecto ARTs to carry out the ADP-ribosylation reaction. This is a similar problem faced by CD38 and CD157, which will be discussed later. The NAD^+ channel connexin 43 therefore could be important for ecto ARTs function as well.[62] One question that is largely ignored in the ecto ARTs publications is whether the ADP-ribosylation reaction is intra-cellular or inter-cellular. Considering that ART-1 and ART-2 are present in T-cells whose function depends on physical contact with other cells, inter-cellular ADP-ribosylation would have important functional implications.

Ecto ARTs are unlikely to modify intracellular proteins because their active sites are out of the cell. However, there are several intracellular proteins that are found to be ADP-ribosylated, including G_β,[63] glutamate dehydrogenase (GDH),[64] and endoplasmic reticulum-resident chaperone GRP78/BiP.[65] Recent evidence suggests that the NAD^+-dependent deacetylases or sirtuins (see the section below) could be the intracellular ARTs responsible for these modifications. For example, mouse SirT6 was found to be self ADP-ribosylated, and no deacetylase substrate has been identified.[66] The more convincing case is the report that mouse SirT4 can ADP-ribosylate and regulate GDH *in vivo*.[67] Other proteins, such as translation elongation factor 2 (eEF-2), have also been reported to be ADP-ribosylated, but it is not clear whether it is truly ADP-ribosylation using NAD^+, or nonspecific glycation with ADP-ribose.[68,69] Questions that need to be addressed are how many intracellular proteins are regulated by ADP-ribosylation/de-ADP-ribosylation (catalyzed by ADP-ribose glycohydrolases), and are there any other protein ADP-ribosyltransferases in addition to ecto ARTs and sirtuins?

NAD^+-dependent deacetylases

Protein acetylation, particularly histone acetylation, is associated with transcriptional activation of genes and is therefore an area of intensive investigation.[70] Histone deacetylation, in contrast, correlates with transcription repression. The first class of histone deacetylases are Zn^{2+}-dependent enzymes that use Zn^{2+} in the active sites to activate water molecules for a nucleophilic attack on the amide bond.[71] The second class of deacetylases, which were only elucidated in the last few years, are NAD^+ dependent and couple NAD^+ hydrolysis to the deacetylation reaction (Fig. 5).

The first NAD^+-dependent deacetylase discovered is yeast Sir2 (silencing information regulator 2), hence these enzymes are collectively termed "sirtuins". Sir2 is required for transcription silencing of specific regions of yeast chromosome, such as the silent mating loci, ribosomal DNA, and telomere.[13] Furthermore, Sir2 is known to be required for yeast life-span extension in a genetic model of calorie restriction, and overexpression of Sir2 can increase yeast life span.[72] However, the molecular mechanisms of silencing and life-span extension were not understood until the enzymatic activity of Sir2 was figured out. In 2000, Guarente and coworkers discovered the ability of Sir2 to deacetylate Histone H3 and H4 in the presence of NAD^+.[73] This finding immediately offered an explanation to the transcription silencing function of Sir2, since histone deacetylation is known to be associated with transcription repression. The discovery by Guarente and coworkers also stimulated studies to understand the enzymatic reaction mechanism of sirtuins. Two labs reported that the reaction products are nicotinamide and acetyl-ADP-ribose, in addition to the deacetylated histones.[74,75] However, the position of the acetyl group on ADP-ribose was not identified, which hinders the elucidation of the reaction mechanism. A few months later, Boeke and Schramm and their coworkers reported their detailed enzymology study of Sir2.[76] NMR, MS, and ^{18}O-labeling of products led to the mechanism shown in Fig. 6A. The key feature of this mechanism is the α-1″-*O*-alkylamidate intermediate formed upon the displacement of nicotinamide by the attack of the acetyl oxygen. The 2″-OH then attacks the intermediate followed by the attack of water to yield the deacetylated protein and 2″-*O*-acetyl ADP-ribose, which can isomerize to 3″-*O*-acetyl ADP-ribose non-enzymatically. Up to today, this remains the generally accepted mechanism. Later work from several labs confirmed this mechanism.[77–81] Several crystal structures of sirtuins, including Sir2 from *Archaeoglobus fulgidus*,[82–84] yeast HST2,[85] human SirT2,[86] and *E. coli* CobB,[87] have been reported, which support the proposed enzymatic reaction mechanism (Fig. 6B).[88–90]

This mechanism alone cannot explain why calorie restriction would induce Sir2-dependent life-span extension. However, it can explain why nicotinamide is an inhibitor of the deacetylation reaction. Since the first step in the mechanism shown in Fig. 6A is reversible, high concentrations of nicotinamide would favor the reverse reaction and inhibit deacetylation.[78,81] This turned out to be the key to understand calorie restriction-induced

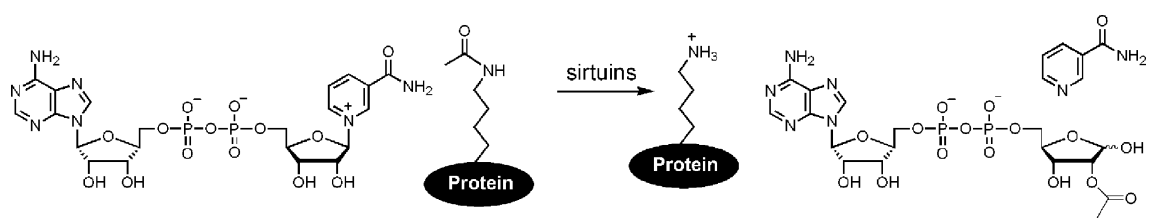

Fig. 5 NAD^+-dependent deacetylation reaction catalyzed by sirtuins. In the reaction, acetyl lysine is converted to free lysine, and NAD^+ is converted to nicotinamide and 2″-acetyl ADP-ribose.

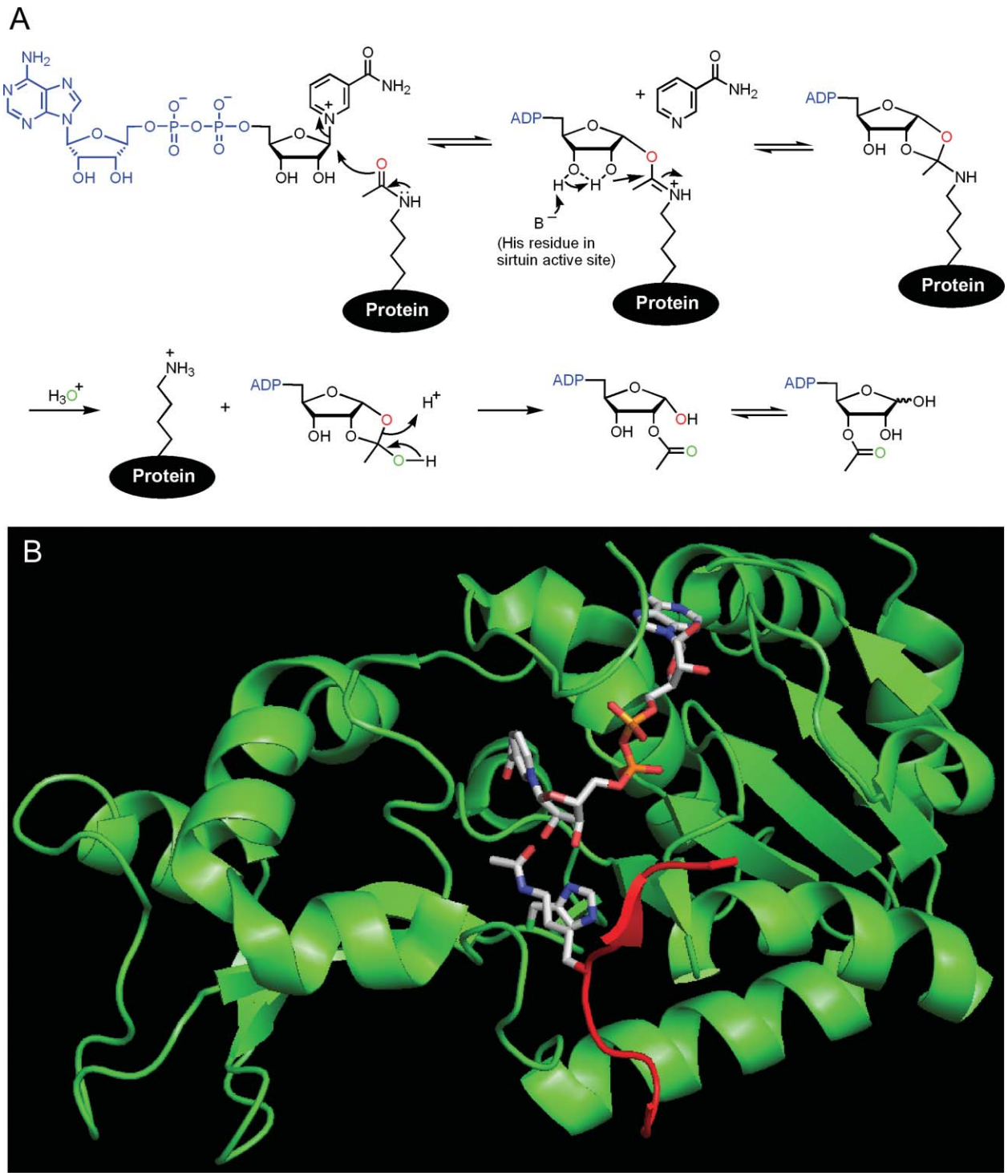

Fig. 6 Mechanism and structure of NAD^+-dependent deacetylases. (A) Sirtuins catalyzed the deacetylation of acetyl lysine residues on substrate proteins. The generally accepted enzymatic reaction mechanism is shown. Some of the oxygen atoms are colored differently to indicate where they come from. (B) Several sirtuin structures have been reported. Shown here is the Sir2 from *A. fulgidus* (PDB 2H4F) in a tertiary complex with NAD^+ and acetylated peptide (red). NAD^+, acetyl lysine residue, and the active site His136 residue are shown in stick representation. In this structure, the acetyl lysine residue is positioned to attack NAD^+ at the anomeric position. The structure supports the mechanism shown in (A) which was derived biochemically.

Sir2-dependent yeast life-span extension. Sinclair and coworkers found that yeast cells upregulate the expression of PNC1 under calorie restriction.[91] PNC1 is the enzyme that converts nicotinamide to nicotinic acid, which is the precursor for NAD^+ biosynthesis in yeast. In contrast to nicotinamide, nicotinic acid is not an inhibitor of Sir2. Therefore, increase in PNC1 expression relieves the nicotinamide inhibition of Sir2, leading to more efficient repression of the ribosomal DNA loci. Repression of the ribosomal DNA loci decreases the production of ribosomal DNA circles *via* recombination, which is one of the major causes of

ageing in yeast, thus leading to yeast life-span extension (Fig. 7). This mechanism is plausible and seems to be accepted by most people in the ageing field. However, calorie restriction-induced life-span may be more complicated and could involve pathways that are sirtuin-independent.[92,93] In addition, the same mechanism is unlikely to be applicable to mammalian cells because mammalian cells use different NAD^+ biosynthetic pathways that do not require the conversion of nicotinamide to nicotinic acid.[2]

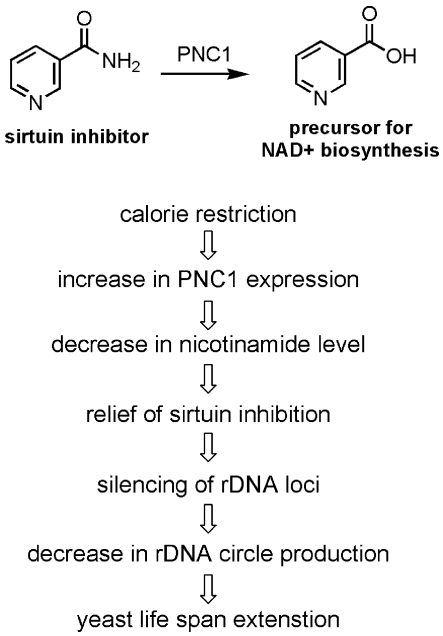

Fig. 7 Sirtuin-dependent mechanism of calorie restriction-induced yeast life-span extension in yeast. Calorie restriction induces the upregulation of PNC1 protein, which converts the sirtuin inhibitor nicotinamide to nicotinic acid, thus relieving the inhibition on sirtuins and ultimately leading to life-span extension.

Sirtuins are evolutionarily conserved from bacteria to mammals. The Salmonella sirtuin CobB can deacetylate the acetylated K609 of acetyl-CoA synthetase to activate its catalytic activity.[94] In yeast there are five sirtuins identified: Sir2, and HST1 to HST4. Humans have seven sirtuins, SirT1 to SirT7.[95] In addition to transcription silencing and ageing, sirtuins are also involved in many other biological processes by deacetylating or ADP-ribosylating other proteins involved in different biological processes. For example, in addition to deacetylation of histones, SirT1 can also remove acetyl groups from transcription factors, such as p53[96] and FOXO,[97,98] SirT2 can deacetylate α-tubulin,[99] SirT3 can deacetylate acetyl-CoA synthetase 2[100,101] and SirT4 can ADP-ribosylate glutamate dehydrogenase.[67] These activities, together with the histone deacetylase activity, suggest sirtuins are important regulators in various biological pathways.[13]

Further studies will be needed to clarify a few issues about sirtuins. One question is what proteins each sirtuin modifies, by either deacetylation or ADP-ribosylation. The biological function of each sirtuin will obviously depend on what protein it modifies and hence regulates. For example, the recent discovery of SirT3 and SirT4 substrates, acetyl-CoA synthetase 2 and glutamate dehydrogenase (GDH), respectively, will lead the study of these two sirtuins to a new level that could not be achieved before. Four of the sirtuins (SirT1, SirT2, SirT3, SirT4) in humans or mice have been associated with at least one substrate protein, while for the rest, no substrate proteins have been identified yet. Even for sirtuins that already have one or more substrate proteins identified, it is possible that they have other unidentified substrate proteins. Once the substrate proteins are identified, the next question is how sirtuin activity (and acetyltransferase activity) is regulated to maintain the correct level of protein acetylation. The best understood regulation mechanism at present is probably by the small molecules NAD^+ and nicotinamide. The discovery of other regulation mechanisms, such as the upregulation of SirT1 expression in response to calorie restriction in mammals,[102] would offer a more complete molecular understanding of sirtuin-dependent biological pathways.

Another unresolved biochemical question is the deacetylation *versus* ADP-ribosylation activity of sirtuins. Although NAD^+-dependent deacetylation is the most robust activity of sirtuins, earlier studies also revealed protein ADP-ribosylation activity. For example, yeast Sir2 was observed to ADP-ribosylate itself, histones, and bovine serum albumin using radio-labeled NAD^+.[103] Whether this ADP-ribosylation activity of Sir2 is physiologically relevant or not is unclear. Mouse SirT6, for which no deacetylation substrate protein has been identified, was reported to have self-ADP-ribosylation activity.[66] But again, the physiological relevance is not clear. Recently, SirT4 is reported to be responsible for the ADP-ribosylation of GDH.[67] The ADP-ribosylation decreases the activity of GDH, and regulates insulin secretion in response to amino acids in mice. The SirT4 example suggests that the ADP-ribosylation activity of other sirtuins might be physiologically relevant, too. Mechanistically, NAD^+-dependent deacetylation is also an ADP-ribosylation process, differing only in the acceptor of the ADP-ribose group (Fig. 8). Can both activities be physiologically significant for all sirtuins? If so, how are the two activities controlled or regulated in the cell? Or do some sirtuins act essentially as deacetylases, while others act mainly as protein ADP-ribosyltransferases? If so, can a sirtuin with mainly deacetylase activity be converted to a sirtuin with mainly protein ADP-ribosyltransferase activity, or *vice versa*? Knowing the answers to these enzymology questions will in turn help to identify the substrate proteins and hence the biological function of sirtuins.

ADP-ribosyltransferases that modify nucleic acids

NAD^+-dependent tRNA 2′-phosphotransferases

In yeast, splicing of tRNA generates tRNA molecules with 2′-phosphate at the splicing junction.[104] Removal of this 2′-phosphate is catalyzed by the NAD^+-dependent 2′-phosphotransferase Tpt1.[105,106] The mechanism of Tpt1 was initially inferred from the study of the *E. coli* protein KptA, which can complement Tpt1 in yeast.[107] Strong evidence has been provided to support a two-step mechanism, shown in Fig. 9. The first step is the transfer of the ADP-ribose group from NAD^+ to the 2′ phosphate of tRNA, forming an ADP-ribosyl tRNA intermediate. For KptA, this intermediate can be isolated, and the isolated intermediate can be converted to product by KptA or Tpt1. The second step is the intramolecular attack on the phosphate by the adjacent 2″-OH, generating ADP-ribose 1″,2″-cyclic phosphate and releasing the mature tRNA. This mechanism is essentially the same as that

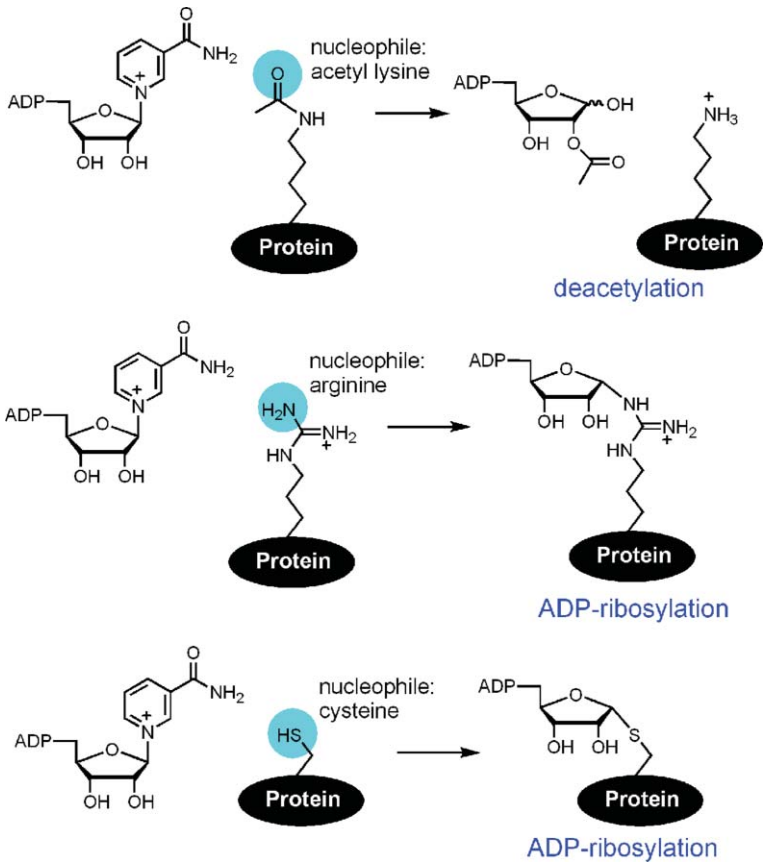

Fig. 8 The deacetylase activity and ADP-ribosyltransferase activity of sirtuins are only different in the nucleophilic residues involved on the substrate proteins. When acetyl Lys is the nucleophile, the reaction is deacetylation. In contrast, when Arg or Cys is the nucleophile, ADP-ribosylated protein would be the end product.

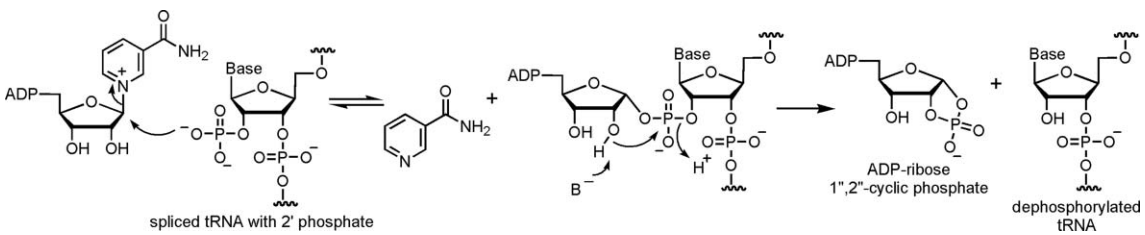

Fig. 9 Proposed reaction mechanism for tRNA 2′-phosphotransferase Tpt1 and KptA. The mechanism in many aspects resembles that of NAD^+-dependent deacetylases.

of NAD^+-dependent deacetylases, differing only by the nature of the nucleophiles involved. For the Tpt1-catalyzed reaction, no ADP-ribosyl tRNA intermediate can be detected. However, a Tpt1 mutant (K69A/R71S) accumulates the intermediate which can be converted to products by wild-type Tpt1.[108] This result suggests that K69 and R71 of Tpt1 are important for the second step of the reaction. Therefore mutation of these two residues significantly slows down the second step, leading to accumulation of the intermediate.

Tpt1 homologs are found in all domains of life—bacteria, archaea, and eukaryotes (including vertebrates).[109] The intriguing thing is that in many species, 2′-phosphate tRNA are not generated because different splicing mechanisms are utilized.[108,110] Therefore, an important question to be addressed is what are the substrates and the biological functions of the Tpt1 homologs in these species.

DNA ADP-ribosylating proteins

The first DNA ADP-ribosylating protein, pierisin-1 was identified from the cabbage butterfly, *Pieris rapae*.[111] It has been shown that pierisin-1 catalyzed the ADP-ribosylation of dG residue in DNA, which is responsible for its cytotoxic activity in mammalian cells. A similar protein, pierisin-2, was identified from another cabbage butterfly species.[112] Pierisins are similar to AB type bacterial toxins, such as diphtheria toxin and cholera toxin. The catalytic domain resides at the N-terminus of pierisin, and the C-terminal fragment is used to target receptor molecules on the host cell surface.[113]

The clam DNA ADP-ribosylating proteins (CARPs) recently identified, however, only contain the catalytic fragment.[114] The function of CARPs is therefore unclear at present.

ADP-ribosyltransferases that modify small molecules

Examples of small molecule ADP-ribosyltransferases include CobT, the bacterial enzyme involved in cobalamin biosynthesis,[115] and the rifampin ADP-ribosyltransferase from certain mycobacteria.[116] CobT can use both NAD^+ and nicotinate mononucleotide (NaMN) as substrates, but the k_{cat}/K_m of CobT for NaMN is much larger than that for NAD^+. However, the *in vivo* concentration of NaMN is very low. Therefore NAD^+ could be a reasonable *in vivo* substrate, making CobT an ADP-ribosyltransferase.[115] The rifampin ADP-ribosyltransferase is responsible for inactivation of the antibiotic rifampin by ADP-ribosylation. However, no detailed biochemical study has been carried out on this enzyme.

Below, the focus of small molecule ADP-ribosyltransferases will be given to a special type of enzymes in mammalian cells, the ADP-ribose cyclases. These enzymes have been the subject of intensive research due to their roles in Ca^{2+} signaling.

ADP-ribosyl cyclases

ADP-ribosyl cyclases,[117] as the name implies, generate cyclic ADP-ribose (cADPR) from NAD^+ (Fig. 10). The first such enzyme was identified as a soluble protein from sea urchin egg homogenate.[118] Mammalian cells so far are known to have two ADP-ribosyl cyclases, CD38 and CD157. Both CD38 and CD157 are ecto enzymes with their active sites outside of the cell membrane. CD38 is a type II membrane protein, tethered to lipid bilayers with a single membrane-spanning helix located at the N-terminus. In contrast, CD157, also called BST-1, is anchored to membranes *via* the covalently linked glycosylphosphotidylinositol at the C-terminus,[119] similar to the ecto ARTs. The structures of all three enzymes have been solved and they look very similar to each other.[120–122]

What's rather unusual about CD38 and CD157 is that they also possess other enzymatic activities, including hydrolysis of cADPR to ADPR and base-exchange reaction of NADP with nicotinic acid to produce nicotinic acid adenine dinucleotide phosphate (NAADP, Fig. 10).[123] These different activities can be explained by the involvement of a common intermediate and the reversibility of the reaction steps involved (Fig. 11A).[124] The intermediate could be covalently attached to the enzyme, since an ADP-ribosylated CD38 intermediate has been trapped with ara-2-F nicotinamide mononucleotide for human CD38.[125] The crystal structure of Aplysia ADP-ribosyl cyclase in a covalent complex with ribose 5-phosphate also supports covalent intermediate formation.[126] The residue on CD38 that is involved in forming the covalent intermediate is Glu226, the position of which is highlighted in the crystal structure of a human CD38 Glu226Gln mutant (Fig. 11B).[125] However, an alternative mechanism which involves the oxocarbenium ion intermediate is also possible (Fig. 11A). A recent crystal structure of human CD38 in complex with NGD^+ captured a structure that looks like the oxocarbenium ion intermediate (Fig. 11C), although it was not determined whether the captured intermediate is capable of undergoing the forward reaction pathway to produce the product or not.[127]

ADP-ribosyl cyclases have attracted a lot of attention in the past decades because their enzymatic reaction products, cADPR and NAADP, are potent second messengers that trigger Ca^{2+} release in cells from internal Ca^{2+} stores.[117,128–130] Furthermore, accumulating evidence suggests that cADPR and NAADP activate different Ca^{2+} stores. cADPR releases Ca^{2+} from the ER similar to inositol triphosphate (IP_3)-triggered Ca^{2+} release, while NAADP releases Ca^{2+} from lysosomal-like organelles.[130,131] It is thought that cADPR-triggered Ca^{2+} release occurs through the rynodine receptor,[132] however, the detailed molecular mechanism (for example, whether cADPR binds directly to the rynodine receptor or not) is not clear.[133] On the other hand, it is not known which receptor NAADP targets,[134] although a partial purification of a potential receptor has been reported.[135] cADPR and NAADP analogs through chemical or chemo-enzymatic synthesis could be useful in isolating and identifying the protein targets that directly interact with cADPR and NAADP and improve our understanding of Ca^{2+} signaling on a molecular level.

The fact that both CD38 and CD157 are ecto enzymes producing cADPR and NAADP on the outside of the cell seems,

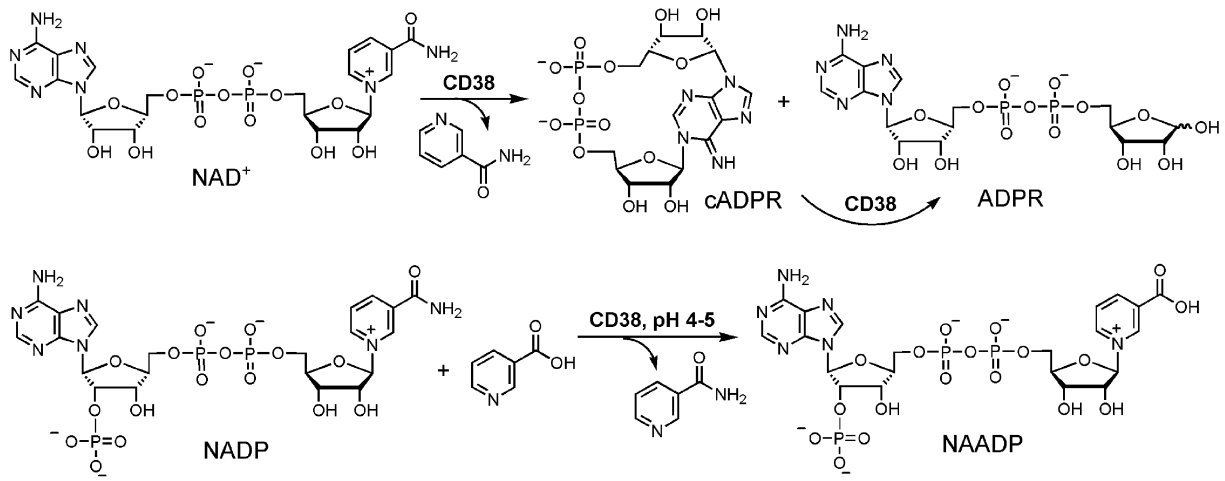

Fig. 10 ADP-ribose cyclase CD38 catalyzes the formation of two Ca^{2+} messengers, cADPR and NAADP.

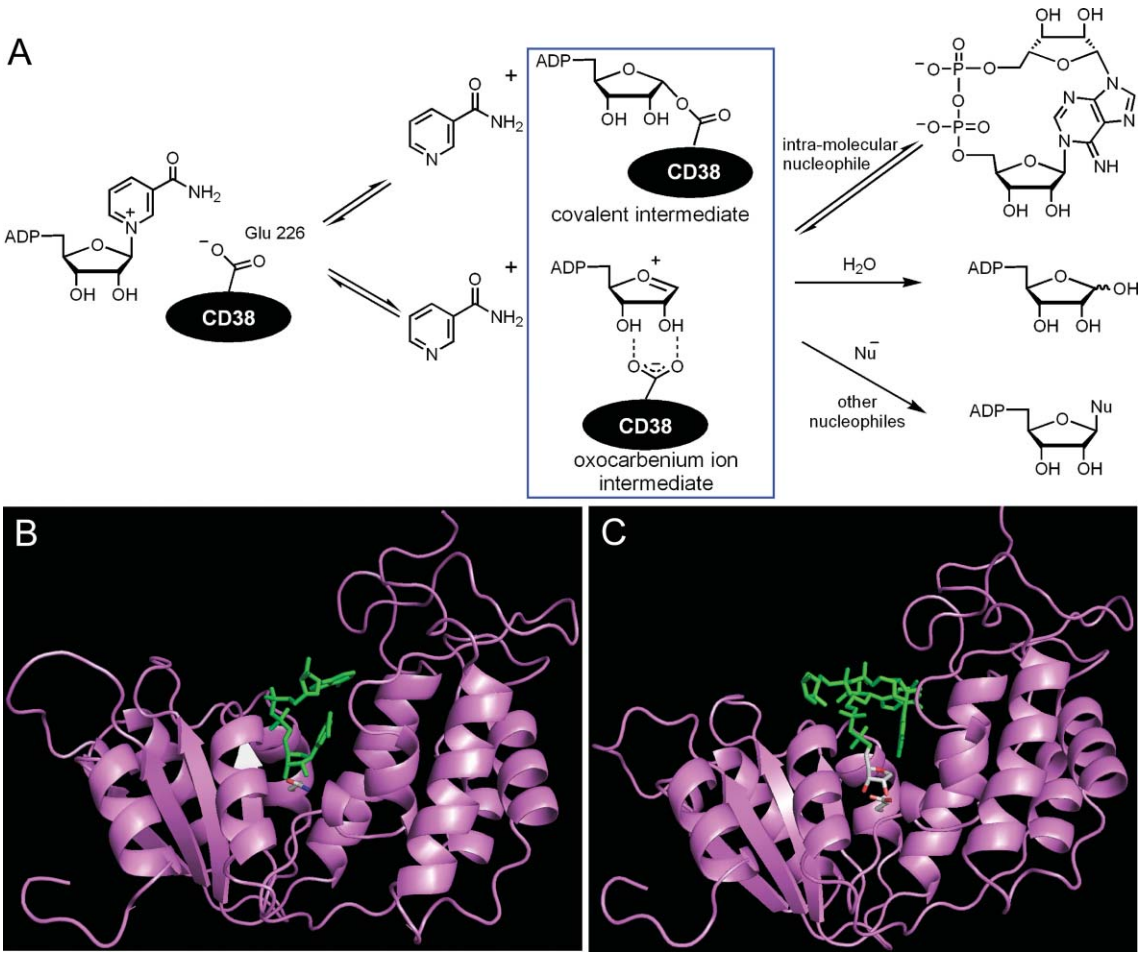

Fig. 11 Proposed enzymatic reaction mechanisms of ADP-ribosyl cyclase CD38. (A) An ADP-ribosyl-enzyme covalent intermediate was proposed (top one in the blue rectangle), which is supported by the isolation of a covalent intermediate when a fluorinated nicotinamide mononucleotide analog was incubated with human CD38. A non-covalent oxocarbenium ion intermediate was also proposed based on an intermediate structure trapped during crystal structure determination, which is shown in (C). (B) Crystal structure of human CD38 Glu226Gln extracellular domain (PDB 2I65, Chain A). The bound NAD^+ molecule (green sticks) and Gln226 residue are shown. (C) Crystal structure of human CD38 soaked with NGD^+ (PDB 2I66, Chain B). In this structure, the nicotinamide is gone, but the ribose 1″ position is not bonded to any residue in CD38 or the guanine part of NGD^+. This intermediate structure is proposed to be the oxocarbenium ion.[127]

at first glance, in conflict with cADPR and NAADP being second messengers functioning inside the cell. If these molecules are indeed produced outside the cell, two questions need to be addressed: are there NAD^+ or $NADP^+$ molecules available outside the cell for the production of cADPR and NAADP by CD38 and CD157, and can cADPR and NAADP get into the cell? It should be pointed out that CD38 is not only present on the cell surface, but also in the membrane of intracellular organelles (such as the nucleus and the endoplasmic reticulum), where the ecto-enzyme domain is actually inward. However, the same topology problem exists even if cADPR and NAADP are produced inside the intracellular organelles. There is evidence to suggest that cells have NAD^+ transporters/channels (such as connexin 43) that can let intracellular NAD^+ out of the cell or extracellular NAD^+ into the cell.[62] Similarly, it was suggested that cADPR and NAADP can go into the cell by specific transporters or *via* CD38.[136–139] There are more puzzling features to the synthesis of NAADP. CD38-catalyzed NAADP production requires acidic pH and high concentration of nicotinic acid.[123] Neither of these requirements would be satisfied in the extracellular fluid or cytoplasma. Therefore, the exact cellular location where NAADP is produced needs to be clarified in the future. If CD38 is the major enzyme responsible for NAADP synthesis, the cellular location must be acidic and have a high nicotinic acid concentration. Alternatively, it is possible that there are other enzymes in the cell capable of catalyzing the formation of NAADP without the requirement of acidic pH or high nicotinic acid concentration.[140]

The biological importance of CD38 enzymatic activity was recently demonstrated in CD38 knock-out mice.[141] Mice lacking CD38 showed defects in maternal nurturing and social behavior due to the decreased secretion of the posterior pituitary hormone oxytocin. The enzymatic activity of CD38 is important for the secretion of oxytocin since the lentiviral expression of wild-type CD38 rescued the defects, but not the expression of a mutant (Arg140Trp) with low enzymatic activity. Although the obvious catalytic mutant Glu226Gln was not tested, other evidence supports the claim that the enzymatic activity is critical for oxytocin secretion. In other cases, whether or not the biological

function of CD38 depends on its enzymatic activity is not clear. For example, CD38 is thought to be important in retinoic acid-induced differentiation of the human leukemia cell HL60[142]. However, this function may be due to other signaling functions of CD38 that are unrelated to enzymatic activity.[143] The other signaling functions of CD38 are still poorly understood at present and are beyond the scope of this manuscript.

Summary and outlook

Nature tends to use a few small molecules (such as ATP, NAD^+, *S*-adenosyl methionine) repeatedly in various biological processes. Here I have briefly summarized a very diverse type of enzyme, ADP-ribosyltransferases, that use NAD^+ as the co-substrate. By modifying a diverse set of substrates, ADP-ribosyltransferases regulate a variety of biological processes and therefore have important biological functions. However, as pointed out in specific sections above, many questions remain to be answered to fully understand the biological functions of these enzymes at a molecular level. In addition, since there is little or no sequence homology among different types of ADP-ribosyltransferases, new members are likely to be identified in the future. The ADP-ribosylation field, therefore, will continue to grow and fuel new discoveries.

The study of ADP-ribosylation will benefit from a multi-disciplinary approach that involves both chemistry and biology. Better analytical tools are needed to detect NAD^+ and its metabolites or biosynthesis precursors, preferably *in vivo* in different cellular locations, and to determine their concentrations. Small molecule inhibitors and NAD^+ analogs will be extremely useful in elucidating the biological functions of ADP-ribosyltransferases. Biochemical and biophysical techniques will be required to study the enzymology and biochemical effects of the enzymatic modification. Ultimately the *in vivo* function will be investigated or confirmed by various biological studies in live cells or animals, including transgenic animals. New technologies, such as RNA interference, DNA chips, and proteomic/metabolomic methods, will certainly speed up the process of discovery in the field. For chemistry students interested in solving biological problems, ADP-ribosylation is an exciting field to be in.

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References

1 F. Berger, M. H. Ramirez-Hernandez and M. Ziegler, *Trends Biochem. Sci.*, 2004, **29**, 111–118.
2 P. O. Hassa, S. S. Haenni, M. Elser and M. O. Hottiger, *Microbiol. Mol. Biol. Rev.*, 2006, **70**, 789–829.
3 I. R. Lehman, *Science*, 1974, **186**, 790–797.
4 J. Y. Lee, C. Chang, H. K. Song, J. Moon, J. K. Yang, H. K. Kim, S. T. Kwon and S. W. Suh, *EMBO J.*, 2000, **19**, 1119–1129.
5 A. Wilkinson, J. Day and R. Bowater, *Mol. Microbiol.*, 2001, **40**, 1241–1248.
6 D. D'Amours, S. Desnoyers, I. D'Silva and G. G. Poirier, *Biochem. J.*, 1999, **342**, 249–268.
7 V. Schreiber, F. Dantzer, J.-C. Ame and G. de Murcia, *Nat. Rev. Mol. Cell Biol.*, 2006, **7**, 517–528.
8 B. Alexander, *Bioessays*, 2001, **23**, 795–806.
9 L. Virag and C. Szabo, *Pharmacol. Rev.*, 2002, **54**, 375–429.
10 D. Corda and M. D. Girolamo, *EMBO J.*, 2003, **22**, 1953–1958.
11 M. Di Girolamo, N. Dani, A. Stilla and D. Corda, *FEBS J.*, 2005, **272**, 4565–4575.
12 I. J. Okazaki and J. Moss, *J. Biol. Chem.*, 1998, **273**, 23617–23620.
13 S. Michan and D. Sinclair, *Biochem. J.*, 2007, **404**, 1–13.
14 A. A. Sauve, C. Wolberger, V. L. Schramm and J. D. Boeke, *Annu. Rev. Biochem.*, 2006, **75**, 435–465.
15 M. Miwa, M. Ishihara, S. Takishima, N. Takasuka, M. Maeda, Z. Yamaizumi, T. Sugimura, S. Yokoyama and T. Miyazawa, *J. Biol. Chem.*, 1981, **256**, 2916–2921.
16 T. Kinoshita, I. Nakanishi, M. Warizaya, A. Iwashita, Y. Kido, K. Hattori and T. Fujii, *FEBS Lett.*, 2004, **556**, 43–46.
17 A. Ruf, V. Rolli, G. de Murcia and G. E. Schulz, *J. Mol. Biol.*, 1998, **278**, 57–65.
18 A. W. Oliver, J.-C. Ame, S. M. Roe, V. Good, G. de Murcia and L. H. Pearl, *Nucleic Acids Res.*, 2004, **32**, 456–464.
19 C. E. Bell and D. Eisenberg, *Biochemistry*, 1996, **35**, 1137–1149.
20 G. Felsenfeld and M. Groudine, *Nature*, 2003, **421**, 448–453.
21 G. G. Poirier, G. D. Murcia, J. Jongstra-Bilen, C. Niedergang and P. Mandel, *Proc. Natl. Acad. Sci. U. S. A.*, 1982, **79**, 3423–3427.
22 G. de Murcia, A. Huletsky, D. Lamarre, A. Gaudreau, J. Pouyet, M. Daune and G. G. Poirier, *J. Biol. Chem.*, 1986, **261**, 7011–7017.
23 F. R. Althaus, *J. Cell Sci.*, 1992, **102**, 663–670.
24 M. E. Bonicalzi, J. F. Haince, A. Droit and G. G. Poirier, *Cell. Mol. Life Sci.*, 2005, **62**, 739–750.
25 K. W. Caldecott, S. Aoufouchi, P. Johnson and S. Shall, *Nucleic Acids Res.*, 1996, **24**, 4387–4394.
26 J.-F. Haince, S. Kozlov, V. L. Dawson, T. M. Dawson, M. J. Hendzel, M. F. Lavin and G. G. Poirier, *J. Biol. Chem.*, 2007, DOI: 10.1074/jbc.M608406200.
27 G. I. Karras, G. Kustatscher, H. R. Buhecha, M. D. Allen, C. Pugieux, F. Sait, M. Bycroft and A. G. Ladurner, *EMBO J.*, 2005, **24**, 1911–1920.
28 G. Kustatscher, M. Hothorn, C. Pugieux, K. Scheffzek and A. G. Ladurner, *Nat. Struct. Mol. Biol.*, 2005, **12**, 624–625.
29 M. Y. Kim, S. Mauro, N. Gevry, J. T. Lis and W. L. Kraus, *Cell*, 2004, **119**, 803–814.
30 D. A. Nusinow, I. Hernandez-Munoz, T. G. Fazzio, G. M. Shah, W. L. Kraus and B. Panning, *J. Biol. Chem.*, 2007, **282**, 12851–12859.
31 M. Cohen-Armon, L. Visochek, D. Rozensal, A. Kalal, I. Geistrikh, R. Klein, S. Bendetz-Nezer, Z. Yao and R. Seger, *Mol. Cell*, 2007, **25**, 297–308.
32 S.-W. Yu, H. Wang, M. F. Poitras, C. Coombs, W. J. Bowers, H. J. Federoff, G. G. Poirier, T. M. Dawson and V. L. Dawson, *Science*, 2002, **297**, 259–263.
33 S.-W. Yu, S. A. Andrabi, H. Wang, N. S. Kim, G. G. Poirier, T. M. Dawson and V. L. Dawson, *Proc. Natl. Acad. Sci. U. S. A.*, 2006, **103**, 18314–18319.
34 A. Tulin and A. Spradling, *Science*, 2003, **299**, 560–562.
35 G. Gradwohl, A. Mazen and G. de Murcia, *Biochem. Biophys. Res. Commun.*, 1987, **148**, 913–919.
36 F. Berger, C. Lau and M. Ziegler, *Proc. Natl. Acad. Sci. U. S. A.*, 2007, **104**, 3765–3770.
37 B.-G. Ju, V. V. Lunyak, V. Perissi, I. Garcia-Bassets, D. W. Rose, C. K. Glass and M. G. Rosenfeld, *Science*, 2006, **312**, 1798–1802.
38 K. Chikamori, J. E. Hill, D. R. Grabowski, E. Zarkhin, A. G. Grozav, S. A. J. Vaziri, J. Wang, A. V. Gudkov, L. R. Rybicki, R. M. Bukowski, A. Yen, M. Tanimoto, M. K. Ganapathi and R. Ganapathi, *Leukemia*, 2006, **20**, 1809–1818.
39 Y. L. Lyu, C.-P. Lin, A. M. Azarova, L. Cai, J. C. Wang and L. F. Liu, *Mol. Cell. Biol.*, 2006, **26**, 7929–7941.
40 P. O. Hassa, C. Buerki, C. Lombardi, R. Imhof and M. O. Hottiger, *J. Biol. Chem.*, 2003, **278**, 45145–45153.
41 J.-C. Amé, C. Spenlehauer and G. de Murcia, *Bioessays*, 2004, **26**, 882–893.
42 S. Smith, I. Giriat, A. Schmitt and T. de Lange, *Science*, 1998, **282**, 1484–1487.
43 S. Smith and T. de Lange, *Curr. Biol.*, 2000, **10**, 1299–1302.

44 P. Chang, M. K. Jacobson and T. J. Mitchison, *Nature*, 2004, **432**, 645–649.
45 P. Chang, M. Coughlin and T. J. Mitchison, *Nat. Cell Biol.*, 2005, **7**, 1133–1139.
46 W. Chang, J. N. Dynek and S. Smith, *Biochem. J.*, 2005, **391**, 177–184.
47 C. T. Walsh, Posttranslational Modification of Proteins: Expanding Nature's Inventory, Roberts and Company Publishers, Englewood, Colorado, 2005.
48 A. Zolkiewska, M. S. Nightingale and J. Moss, *Proc. Natl. Acad. Sci. U. S. A.*, 1992, **89**, 11352–11356.
49 F. Koch-Nolte, F. Haag, R. Braren, M. Kuhl, J. Hoovers, S. Balasubramanian, F. Bazan and H.-G. Thiele, *Genomics*, 1997, **39**, 370–376.
50 G. Glowacki, R. Braren, K. Firner, M. Nissen, M. Kuhl, P. Reche, F. Bazan, M. Cetkovic-Cvrlje, E. Leiter, F. Haag and F. Koch-Nolte, *Protein Sci.*, 2002, **11**, 1657–1670.
51 A. Zolkiewska and J. Moss, *J. Biol. Chem.*, 1993, **268**, 25273–25276.
52 Z. F. Zhao, J. Gruszczynska-Biegala and A. Zolkiewska, *Biochem. J.*, 2005, **385**, 309–317.
53 A. Zolkiewska and J. Moss, *J. Biol. Chem.*, 1995, **270**, 9227–9233.
54 S. Okamoto, O. Azhipa, Y. Yu, E. Russo and G. Dennert, *J. Immunol.*, 1998, **160**, 4190–4198.
55 Z.-X. Liu, Y. Yu and G. Dennert, *J. Biol. Chem.*, 1999, **274**, 17399–17401.
56 G. Paone, A. Wada, L. A. Stevens, A. Matin, T. Hirayama, R. L. Levine and J. Moss, *Proc. Natl. Acad. Sci. U. S. A.*, 2002, **99**, 8231–8235.
57 E. M. Jones and A. Baird, *Biochem. J.*, 1997, **323**, 173–177.
58 B. A. Saxty, M. Yadollahi-Farsani, P. D. Upton, S. R. Johnstone and J. MacDermot, *Br. J. Pharmacol.*, 2001, **133**, 1219–1226.
59 S. Adriouch, W. Ohlrogge, F. Haag, F. Koch-Nolte and M. Seman, *J. Immunol.*, 2001, **167**, 196–203.
60 M. Seman, S. Adriouch, F. Scheuplein, C. Krebs, D. Freese, G. Glowacki, P. Deterre, F. Haag and F. Koch-Nolte, *Immunity*, 2003, **19**, 571–582.
61 C. Krebs, W. Koestner, M. Nissen, V. Welge, I. Parusel, F. Malavasi, E. H. Leiter, R. M. Santella, F. Haag and F. Koch-Nolte, *Anal. Biochem.*, 2003, **314**, 108–115.
62 S. Bruzzone, L. Guida, E. Zocchi, L. Franco and A. De Flora, *FASEB J.*, 2001, **15**, 10–12.
63 R. Lupi, D. Corda and M. Di Girolamo, *J. Biol. Chem.*, 2000, **275**, 9418–9424.
64 A. Herrero-Yraola, S. M. A. Bakhit, P. Franke, C. Weise, M. Schweiger, D. Jorcke and M. Ziegler, *EMBO J.*, 2001, **20**, 2404–2412.
65 G. H. Leno and B. E. Ledford, *FEBS J.*, 1990, **276**, 29–33.
66 G. Liszt, E. Ford, M. Kurtev and L. Guarente, *J. Biol. Chem.*, 2005, **280**, 21313–21320.
67 M. C. Haigis, R. Mostoslavsky, K. M. Haigis, K. Fahie, D. C. Christodoulou, A. J. Murphy, D. M. Valenzuela, G. D. Yancopoulos, M. Karow, G. Blander, C. Wolberger, T. A. Prolla, R. Weindruch, F. W. Alt and L. Guarente, *Cell*, 2006, **126**, 941–954.
68 J. L. Fendrick and W. J. Iglewski, *Proc. Natl. Acad. Sci. U. S. A.*, 1989, **86**, 554–557.
69 M. Bektas, H. Akcakaya, A. Aroymak, R. Nurten and E. Bermek, *Int. J. Biochem. Cell Biol.*, 2005, **37**, 91.
70 S. Y. Roth, J. M. Denu and C. D. Allis, *Annu. Rev. Biochem.*, 2001, **70**, 81–120.
71 C. M. Grozinger and S. L. Schreiber, *Chem. Biol.*, 2002, **9**, 3–16.
72 M. Kaeberlein, M. McVey and L. Guarente, *Genes Dev.*, 1999, **13**, 2570–2580.
73 S.-i. Imai, C. M. Armstrong, M. Kaeberlein and L. Guarente, *Nature*, 2000, **403**, 795–800.
74 K. G. Tanner, J. Landry, R. Sternglanz and J. M. Denu, *Proc. Natl. Acad. Sci. U. S. A.*, 2000, **97**, 14178–14182.
75 J. C. Tanny and D. Moazed, *Proc. Natl. Acad. Sci. U. S. A.*, 2001, **98**, 415–420.
76 A. A. Sauve, I. Celic, J. Avalos, H. Deng, J. D. Boeke and V. L. Schramm, *Biochemistry*, 2001, **40**, 15456–15463.
77 M. D. Jackson and J. M. Denu, *J. Biol. Chem.*, 2002, **277**, 18535–18544.
78 M. D. Jackson, M. T. Schmidt, N. J. Oppenheimer and J. M. Denu, *J. Biol. Chem.*, 2003, **278**, 50985–50998.
79 B. C. Smith and J. M. Denu, *Biochemistry*, 2006, **45**, 272–282.
80 B. C. Smith and J. M. Denu, *J. Am. Chem. Soc.*, 2007, **129**, 5802–5803.
81 A. A. Sauve and V. L. Schramm, *Biochemistry*, 2003, **42**, 9249–9256.
82 J. L. Avalos, I. Celic, S. Muhammad, M. S. Cosgrove, J. D. Boeke and C. Wolberger, *Mol. Cell*, 2002, **10**, 523–535.
83 J.-H. Chang, H.-C. Kim, K.-Y. Hwang, J.-W. Lee, S. P. Jackson, S. D. Bell and Y. Cho, *J. Biol. Chem.*, 2002, **277**, 34489–34498.
84 J. Min, J. Landry, R. Sternglanz and R.-M. Xu, *Cell*, 2001, **105**, 269–279.
85 K. Zhao, X. Chai and R. Marmorstein, *Structure*, 2003, **11**, 1403–1411.
86 M. S. Finnin, J. R. Donigian and N. P. Pavletich, *Nat. Struct. Mol. Biol.*, 2001, **8**, 621–625.
87 K. Zhao, X. Chai and R. Marmorstein, *J. Mol. Biol.*, 2004, **337**, 731–741.
88 K. G. Hoff, J. L. Avalos, K. Sens and C. Wolberger, *Structure*, 2006, **14**, 1231–1240.
89 B. D. Sanders, K. Zhao, J. T. Slama and R. Marmorstein, *Mol. Cell*, 2007, **25**, 463–472.
90 K. Zhao, R. Harshaw, X. Chai and R. Marmorstein, *Proc. Natl. Acad. Sci. U. S. A.*, 2004, **101**, 8563–8568.
91 R. M. Anderson, K. J. Bitterman, J. G. Wood, O. Medvedik and D. A. Sinclair, *Nature*, 2003, **423**, 181–185.
92 M. Kaeberlein, R. W. Powers, III, K. K. Steffen, E. A. Westman, D. Hu, N. Dang, E. O. Kerr, K. T. Kirkland, S. Fields and B. K. Kennedy, *Science*, 2005, **310**, 1193–1196.
93 P. Fabrizio, C. Gattazzo, L. Battistella, M. Wei, C. Cheng, K. McGrew and V. D. Longo, *Cell*, 2005, **123**, 655–667.
94 V. J. Starai, I. Celic, R. N. Cole, J. D. Boeke and J. C. Escalante-Semerena, *Science*, 2002, **298**, 2390–2392.
95 R. A. Frye, *Biochem. Biophys. Res. Commun.*, 2000, **273**, 793–798.
96 H. Vaziri, S. K. Dessain, E. N. Eaton, S.-I. Imai, R. A. Frye, T. K. Pandita, L. Guarente and R. A. Weinberg, *Cell*, 2001, **107**, 149–159.
97 A. Brunet, L. B. Sweeney, J. F. Sturgill, K. F. Chua, P. L. Greer, Y. Lin, H. Tran, S. E. Ross, R. Mostoslavsky, H. Y. Cohen, L. S. Hu, H.-L. Cheng, M. P. Jedrychowski, S. P. Gygi, D. A. Sinclair, F. W. Alt and M. E. Greenberg, *Science*, 2004, **303**, 2011–2015.
98 M. C. Motta, N. Divecha, M. Lemieux, C. Kamel, D. Chen, W. Gu, Y. Bultsma, M. McBurney and L. Guarente, *Cell*, 2004, **116**, 551–563.
99 B. J. North, B. L. Marshall, M. T. Borra, J. M. Denu and E. Verdin, *Mol. Cell*, 2003, **11**, 437–444.
100 W. C. Hallows, S. Lee and J. M. Denu, *Proc. Natl. Acad. Sci. U. S. A.*, 2006, **103**, 10230–10235.
101 B. Schwer, J. Bunkenborg, R. O. Verdin, J. S. Andersen and E. Verdin, *Proc. Natl. Acad. Sci. U. S. A.*, 2006, **103**, 10224–10229.
102 H. Y. Cohen, C. Miller, K. J. Bitterman, N. R. Wall, B. Hekking, B. Kessler, K. T. Howitz, M. Gorospe, R. de Cabo and D. A. Sinclair, *Science*, 2004, **305**, 390–392.
103 J. C. Tanny, G. J. Dowd, J. Huang, H. Hilz and D. Moazed, *Cell*, 1999, **99**, 735–745.
104 J. Abelson, C. R. Trotta and H. Li, *J. Biol. Chem.*, 1998, **273**, 12685–12688.
105 G. M. Culver, S. M. McCraith, S. A. Consaul, D. R. Stanford and E. M. Phizicky, *J. Biol. Chem.*, 1997, **272**, 13203–13210.
106 S. L. Spinelli, S. A. Consaul and E. M. Phizicky, *RNA*, 1997, **3**, 1388–1400.
107 S. L. Spinelli, R. Kierzek, D. H. Turner and E. M. Phizicky, *J. Biol. Chem.*, 1999, **274**, 2637–2644.
108 M. A. Steiger, J. E. Jackman and E. M. Phizicky, *RNA*, 2005, **11**, 99–106.
109 S. L. Spinelli, H. S. Malik, S. A. Consaul and E. M. Phizicky, *Proc. Natl. Acad. Sci. U. S. A.*, 1998, **95**, 14136–14141.
110 F. A. Laski, A. Z. Fire, U. L. RajBhandary and P. A. Sharp, *J. Biol. Chem.*, 1983, **258**, 11974–11980.
111 T. Takamura-Enya, M. Watanabe, Y. Totsuka, T. Kanazawa, Y. Matsushima-Hibiya, K. Koyama, T. Sugimura and K. Wakabayashi, *Proc. Natl. Acad. Sci. U. S. A.*, 2001, **98**, 12414–12419.
112 T. Takamura-Enya, M. Watanabe, K. Koyama, T. Sugimura and K. Wakabayashi, *Biochem. Biophys. Res. Commun.*, 2004, **323**, 579–582.
113 Y. Matsushima-Hibiya, M. Watanabe, K. I. P. J. Hidari, D. Miyamoto, Y. Suzuki, T. Kasama, T. Kanazawa, K. Koyama, T. Sugimura and K. Wakabayashi, *J. Biol. Chem.*, 2003, **278**, 9972–9978.
114 T. Nakano, Y. Matsushima-Hibiya, M. Yamamoto, S. Enomoto, Y. Matsumoto, Y. Totsuka, M. Watanabe, T. Sugimura and K. Wakabayashi, *Proc. Natl. Acad. Sci. U. S. A.*, 2006, **103**, 13652–13657.
115 L. A. Maggio-Hall and J. C. Escalante-Semerena, *Microbiology*, 2003, **149**, 983–990.
116 S. Quan, T. Imai, Y. Mikami, K. Yazawa, E. R. Dabbs, N. Morisaki, S. Iwasaki, Y. Hashimoto and K. Furihata, *Antimicrob. Agents Chemother.*, 1999, **43**, 181–184.

117 H. C. Lee, *Annu. Rev. Pharmacol. Toxicol.*, 2001, **41**, 317–345.
118 H. C. Lee and R. Aarhus, *Cell Regul.*, 1991, **2**, 203–209.
119 T. Kaisho, J. Ishikawa, K. Oritani, J. Inazawa, H. Tomizawa, O. Muraoka, T. Ochi and T. Hirano, *Proc. Natl. Acad. Sci. U. S. A.*, 1994, **91**, 5325–5329.
120 G. S. Prasad, D. E. McRee, E. A. Stura, D. G. Levitt, H. C. Lee and C. D. Stout, *Nat. Struct. Biol.*, 1996, **3**, 957–964.
121 S. Yamamoto-Katayama, M. Ariyoshi, K. Ishihara, T. Hirano, H. Jingami and K. Morikawa, *J. Mol. Biol.*, 2002, **316**, 711–723.
122 Q. Liu, I. A. Kriksunov, R. Graeff, C. Munshi, H. C. Lee and Q. Hao, *Structure*, 2005, **13**, 1331–1339.
123 R. Aarhus, R. M. Graeff, D. M. Dickey, T. F. Walseth and H. C. Lee, *J. Biol. Chem.*, 1995, **270**, 30327–30333.
124 A. A. Sauve, C. Munshi, H. C. Lee and V. L. Schramm, *Biochemistry*, 1998, **37**, 13239–13249.
125 A. A. Sauve, H. Deng, R. H. Angeletti and V. L. Schramm, *J. Am. Chem. Soc.*, 2000, **122**, 7856–7859.
126 M. L. Love, D. M. E. Szebenyi, I. A. Kriksunov, D. J. Thiel, C. Munshi, R. Graeff, H. C. Lee and Q. Hao, *Structure*, 2004, **12**, 477–486.
127 Q. Liu, I. A. Kriksunov, R. Graeff, C. Munshi, H. C. Lee and Q. Hao, *J. Biol. Chem.*, 2006, **281**, 32861–32869.
128 H. C. Lee and R. Aarhus, *J. Biol. Chem.*, 1995, **270**, 2152–2157.
129 H. C. Lee, R. Aarhus and T. F. Walseth, *Science*, 1993, **261**, 352–355.
130 H. C. Lee, *J. Biol. Chem.*, 2005, **280**, 33693–33696.
131 M. Yamasaki, G. C. Churchill and A. Galione, *FEBS J.*, 2005, **272**, 4598–4606.
132 A. Galione, H. C. Lee and W. B. Busa, *Science*, 1991, **253**, 1143–1146.
133 T. F. Walseth, R. Aarhus, J. A. Kerr and H. C. Lee, *J. Biol. Chem.*, 1993, **268**, 26686–26691.
134 A. Galione and M. Ruas, *Cell. Calcium*, 2005, **38**, 273–280.
135 R. A. Billington, J. Bak, A. Martinez-Coscolla, M. Debidda and A. A. Genazzani, *Br. J. Pharmacol.*, 2004, **142**, 1241–1246.
136 L. Franco, L. Guida, S. Bruzzone, E. Zocchi, C. Usai and A. D. Flora, *FASEB J.*, 1998, **12**, 1507–1520.
137 L. Guida, S. Bruzzone, L. Sturla, L. Franco, E. Zocchi and A. De Flora, *J. Biol. Chem.*, 2002, **277**, 47097–47105.
138 L. Guida, L. Franco, S. Bruzzone, L. Sturla, E. Zocchi, G. Basile, C. Usai and A. De Flora, *J. Biol. Chem.*, 2004, **279**, 22066–22075.
139 R. A. Billington, E. A. Bellomo, E. M. Floriddia, J. Erriquez, C. Distasi and A. A. Genazzani, *FASEB J.*, 2006, **20**, 521–523.
140 S. Soares, M. Thompson, T. White, A. Isbell, M. Yamasaki, Y. Prakash, F. E. Lund, A. Galione and E. N. Chini, *Am. J. Physiol.: Cell Physiol.*, 2007, **292**, C227–239.
141 D. Lin, H.-X. Liu, H. Hirai, T. Torashima, T. Nagai, O. Lopatina, N. A. Shnayder, K. Yamada, M. Noda, T. Seike, K. Fujita, S. Takasawa, S. Yokoyama, K. Koizumi, Y. Shiraishi, S. Tanaka, M. Hashii, T. Yoshihara, K. Higashida, M. S. Islam, N. Yamada, K. Hayashi, N. Noguchi, I. Kato, H. Okamoto, A. Matsushima, A. Salmina, T. Munesue, N. Shimizu, S. Mochida, M. Asano and H. Higashida, *Nature*, 2007, **446**, 41–45.
142 C. B. Munshi, R. Graeff and H. C. Lee, *J. Biol. Chem.*, 2002, **277**, 49453–49458.
143 F. E. Lund, *Mol. Med.*, 2006, **12**, 328–333.

